

HEREDITARY INFECTION, WITH
SPECIAL REFERENCE TO ITS
OCCURRENCE IN *HYALOMMA*
AEGYPTIUM INFECTED WITH
CRITHIDIA HYALOMMAE

BY

CAPTAIN W. R. O'FARRELL, R.A.M.C.

PROTOZOOLOGIST TO THE WELLCOME TROPICAL RESEARCH LABORATORIES, KHARTOUM

(Received for publication 8 October, 1913)

PLATES XXXVIII—XL

INTRODUCTION

Our knowledge of the rôle played by the blood-sucking parasites as transmitters of human and animal diseases has considerably advanced during recent years. Consequently, the study of these vehicles of transmission has increased, and many cases of hyper-parasitism have been discovered. During the search for the pathogenic varieties others have been encountered, and these, though their pathogenicity be confined only to their insect hosts, must not be overlooked.

In three ways, a comprehensive study of their life cycles is of value: Firstly, in order to prevent their stages from being confused with the stages in the life histories of more important parasites. Secondly, to act as a guide to the possible behaviour of the disease-producing varieties, and thirdly, as a weapon of offence against their parasitic hosts.

Probably the most important stage in the life cycle of all hyper-parasites is the hereditary infection stage. Hereditary infection does not deal with individuals but with generations, and this method of infection is a higher grade of organisation and of evolution than simpler methods of infection. The complete life cycles of but few hereditary infections are known, though a considerable number of hereditary infection-transmissions are known to exist.

Consequently any addition to our knowledge of such infections is of value. Moreover, any example that can be studied with any degree of facility is worthy of note. The hereditary infection of *Hyalomma aegyptium* by *Crithidia hyalommae* is one of these, and will be described in the following pages.

HISTORICAL

The occurrence of hereditary infection has been suggested, observed and worked out in the insect flagellates already, and among the authors who advanced this line of research I have primarily to mention Miss A. Porter, whose work on *Crithidia melophagia*, Flu, 1908, has proved of valuable assistance. As early as 1903 Dr. L. W. Sambon put forward the hypothesis of the possibility of the occurrence of hereditary infection of the tsetse fly by *Trypanosoma gambiense*.

P. C. Flu (1908) published an account of a flagellate parasitic in the ovaries and small larval forms of the sheep-kek, *Melophagus ovinus*, Linnaeus.

L. D. Swingle (1909) working in Nebraska, described rounded and plasmodial stages of the same parasite, occurring in the egg of *Melophagus ovinus*.

A. Porter (1910) described the intestinal contaminative method of infection and the full cycle and mode of hereditary infection of the same parasite in the host, egg, developing embryo, and fully hatched *Melophagus*.

In a preliminary note published in August, 1913, I have given a brief description of the flagellate stage and discussed the movements of *Crithidia hyalommae*, O'Farrell, 1913. The movements will not therefore be further described, except the special movement connected with the entry of the flagellate into the ovarian system of the tick. The life cycle passed in the larva and nymph will form the subject of a future communication.

THEORETICAL APPLICATIONS

Apart from the practical proofs of the hereditary transmission of *Crithidia hyalommae* in *Hyalomma aegyptium*, certain theoretical conclusions formed the basis of the search for such proofs. These conclusions were:—

1. *The infection was not inoculative*, i.e., derived from the vertebrate host, because:—

(a) All the ticks collected from one given cow were not infected.

(b) Ticks other than *Hyalomma aegyptium*, although among batches of infected ticks, never showed infection.

(c) The cows from which the infected ticks were collected were invariably healthy.

2. *The infection was not contaminative*, because:—

(a) Ticks, unlike other blood-sucking parasites generally, do not move about much on their hosts (except the males), but settle in one place and slowly secure an attachment, where they remain till replete.

(b) The natural secretions and excretions dry very rapidly, and are often voided on the earth after the tick detaches itself from its host.

3. *The infection was hereditary*, because:—

(a) The height of crithidial infection occurred about the time of oviposition.

(b) The occurrence of the infection in batches of ticks all of which probably belong to the same generation.

The practical application of these lines of thought worked out as follows:—

1. The intestinal diverticula of the ticks were never found to harbour parasites.
2. The haemocoelic fluid was the habitat of the early pre-flagellate forms.
3. These early pre-flagellate forms developed into full grown Crithidia in the haemocoelic fluid. When these were present in large numbers they were found to migrate to the ovarian system.
4. The hereditary infecting forms were found entering the ova.
5. Plasmodial forms were found in the deposited eggs.

MATERIAL AND METHODS

Many specimens of *Hyalomma aegyptium* were examined from different localities, but always from cows, which are their common hosts. Some batches of ticks never showed infection, while in others infection was the rule.

To study the living organism, living specimens were examined with ordinary light and with dark ground illumination, but with the latter the Crithidia were found to degenerate and die rapidly because of the strong light and heat produced by the source of illumination used in working with a paraboloid condenser. Vital staining was also employed.

To obtain the haemocoelic fluid a portion of any of the legs is removed with a fine pair of scissors. For stained specimens of tissues all ticks were carefully dissected under a dissecting microscope, and the organs after carefully washing in saline solution, as recommended by Sir William Leishman, were smeared out on slides.

Osmic acid and alcohol were employed as fixatives, and also special methods of wet fixation were used, such as Schaudinn's fluid (corrosive-acetic-alcohol), and Bouin's fluid (picro-formol-acetic-alcohol).

Various stains were used—Leishman's and Giemsa's solutions gave good results. Bohmer's haematoxylin (which in this climate is rather superior to Delafield's haematoxylin) and Heidenhain's iron haematoxylin and its modifications were employed.

Cultivation of the flagellate has proved negative up to the present, together with attempts to inoculate animals. Frequent examinations and cultivation of the blood of the cows have given negative results.

DISTRIBUTION OF THE PARASITE IN THE HOST

The majority of insect flagellates are found parasitic in the alimentary tracts of their hosts, but in this respect the Crithidia of *Hyalomma aegyptium* is an exception. The haemocoelic fluid is the seat of election for the development and subsequent distribution of the parasite in the adult tick.

Shortly after removal of the ticks from their hosts (cattle in this instance) the early pre-flagellate forms may be found in the haemocoelic fluid (Pl. XXXVIII, figs. 1-5), which is a dark amber colour, and is of about the consistency of human blood serum. Microscopically it is seen to contain among other elements the large vacuolated haemocoelic corpuscles. The nuclear and protoplasmic portions of these cells, when coloured with Leishman's or Giemsa's

solutions, show a tendency to stain, as do the nuclei, blepharoplasts and protoplasm of human and animal parasites. This point is of importance, as fragments of these cells might easily be mistaken at first sight for parasites.

A few days after the ticks have been collected, the early pre-flagellate forms show evidences of division and increase in number (figs. 5, 6, 8).

After about four to ten days, that is, a little time previous to oviposition, early flagellate forms make their appearance (figs. 11-18). Rapid multiplication of the young flagellates now takes place, with the formation of division rosettes (figs. 18 and 19), and the growth to adult *Crithidia* (Pl. XXXVIII, figs. 20-29; Pl. XXXIX, figs. 31-33) soon follows:—

The fully-grown *Crithidia* multiply by longitudinal division (Pl. XXXIX, figs. 30, 34-39) till the smallest drop of haemocoelic fluid presents a felted mass of crithidial bodies and waving flagella. Owing to the great numbers of parasites present, the haemocoelic fluid is a milk-white colour. Towards the end of oviposition, and just previous to the death of the tick, post-flagellate forms may be found (figs. 40-44) in the haemocoelic fluid, although in some cases all forms disappear from it. The post-flagellate forms also occur in the genital tract, and are perhaps better termed ovarian forms: they will be dealt with under the description of hereditary infection.

The intestinal diverticula and malpighian tubes have not been found to harbour any of the parasites in the adult tick. Sometimes early division forms (Pl. XL, fig. 62) are found in the salivary glands, but this seems to be the exception rather than the rule.

The parasites were found more commonly in female than in male ticks.

MORPHOLOGY

The life cycle of *Crithidia hyalommae* in the adult tick may, for the sake of description, be divided into four periods which, however, merge into each other. These are the pre-flagellate, the flagellate, the post-flagellate, and ovarian stages. The morphology of the ovarian stage will be dealt with under a subsequent heading. The morphology of the pre-flagellate, the flagellate and post-flagellate stages may be described as follows:—

THE PRE-FLAGELLATE STAGE

The early pre-flagellate stages of *C. hyalommae* found in the haemocoelic fluid are rounded or oval Leishmania-like bodies (Pl. XXXVIII, figs. 1-5, 7, 8), which vary from 4 to 12 μ in length and from 4 to 11 μ in breadth. The protoplasm of these forms is of a finely reticulate nature, containing distinct vacuoles (fig. 4) and a rounded nucleus which is situated near the periphery of the parasite (figs. 1, 2). The blepharoplast is rounded or bow shaped (figs. 1, 2), and acquires the characteristic dark tint of such structures when coloured by the various stains. It may be placed at the side of, or opposite to, the nucleus (figs. 3, 4).

These early forms soon show evidences of division (fig. 5), two daughter parasites resulting, smaller in size and with more granular protoplasm than their precursors. Plasmodial forms (fig. 6) may result from rapid division. Increase in the size of the flagellate bodies follows and the flagellum makes its appearance, arising in the neighbourhood of the blepharoplast. The flagellum in its outward growth carries with it a portion of the periplast which forms the undulating membrane.

After the formation of the flagellum rapid division takes place, rosettes being formed (figs. 18, 19), indeed nearly all stages from rounded flagellates to nearly adult Crithidia may be seen in the same specimen and in the same rosette (figs. 18, 19, 27). It may also be noted that individual members of a division rosette grow at unequal rates. The aflagellar end now grows outwards, at first wide and blunted (figs. 25, 26, 29), but it soon narrows down and tapers off into a fine point (figs. 27, 28).

THE FLAGELLATE STAGE

The fully-grown flagellates present the crithidial type of body, but are unusually long and slender (Pl. XXXIX, figs. 28a-36). The length of the body and flagellum varies from 26 μ to 50 μ , the length of the flagellum alone being 12.5 μ . The average breadth of the nucleus varies from 2 μ to 2.5 μ .

The aflagellar extremity tapers gradually to a fine point, while the flagellate end of the body is continued for some distance along the flagellum.

The flagellum, taking its origin from the vicinity of the blepharoplast, runs along the free border of the undulating membrane until the extremity of the body is reached. Here it escapes from the periplast and projects freely for a considerable distance. The flagellum is never connected with the blepharoplast. A basal granule has been noted (fig. 31), but its presence is not always evident, probably because of its close relation to the blepharoplast. The undulating membrane is well marked, and in living specimens its movements may be observed. In stained preparations it may be seen thrown into folds.

The nucleus is roundish (figs. 28a-36) or oval, and generally lies about the middle of the body, the whole breadth of which it nearly occupies (figs. 28a-36). The structure of the nucleus varies. At times the nucleus appears somewhat vesicular with a marked karyosome (figs. 31-33); at other times the chromatin of the nucleus is distributed in granules within the nuclear membrane (fig. 30). When division is about to take place, the nuclear material is generally more granular (fig. 30).

The blepharoplast of *C. hyalommae* is well defined in all stained specimens, and colours deeply. Its position varies within what might be termed the limits of crithidial definition (Patton, 1908). It may be closely applied to or a little distance from the flagellar or aflagellar side of the nucleus (figs. 28a-33).

In all cases the flagellum arises in the close vicinity of the blepharoplast, in whatever position the latter may be situated. Two blepharoplasts may often be observed (figs. 30 and 35-38), but this is really an early division phase. The blepharoplast is usually rod-shaped (fig. 28a), and sometimes shows a diplosome appearance (fig. 34), probably indicative of approaching division.

Chromatoid granules may be present in the protoplasm. In fresh specimens the protoplasm of the flagellar extremity of the body is markedly granular.

THE POST-FLAGELLATE STAGE

The post-flagellate forms seen in the haemocoelic fluid are merely rounding-up parasites (Pl. XXXIX, figs. 40-44). They are similar to the ovarian forms which undergo the same process in

the walls of the female generative tract, and which will be described when dealing with the hereditary infection.

DIVISION

In the pre-flagellate stages division is initiated by the blepharoplast, which divides into two nearly equal masses. These masses now move one on either side of the nucleus (Pl. XXXVIII, fig. 5), or form the base of a triangle with the nucleus at the apex (fig. 8). The nucleus now divides into two, and when the binucleate system is completed the protoplasm splits, two daughter forms resulting. Large plasmodial forms may be produced in the haemocoelic fluid before the flagella are formed (fig. 6).

In the early flagellate stages, the same phenomena are observed; the blepharoplast is often bow-shaped before splitting (fig. 11). The flagellum divides, commencing at its blepharoplastic end (fig. 11) with the division of the blepharoplast and basal granule. The blepharoplast and flagellum may have divided, and protoplasmic cleavage may have commenced before the nucleus has completed its division (fig. 15). The lashing movements of the flagella at this stage may assist in this early protoplasmic division. Fig. 15 shows this condition, with details of nuclear division: the nucleus still shows the mantle fibres surrounding the centrodosome which connects the equatorial plates.

Division in the early flagellates is rapid, and division rosettes are formed (figs. 18, 19). Two or more phases in division may be observed in the same rosette (figs. 18, 19).

In the full-grown *Crithidia* longitudinal division takes place. Before division the blepharoplast presents a diplosome appearance (Pl. XXXIX, fig. 34), and divides first, with the flagellum (fig. 35). One portion of the blepharoplast and its future flagellum migrates to the opposite side of the nucleus. The blepharoplasts may be connected together by a thin chromatin line (fig. 36).

The nucleus, which is generally granular at this stage, divides into two. The two flagellar ends separate, and the flagella, moving rapidly, separate the remainder of the organism into two similar adult *Crithidia*.

Division of the post-flagellate stages may occur in the haemo-

coelic fluid, but takes place to a marked extent in the ovarian tissues (Pl. XXXIX, figs. 51, 52), ovarian cells (Pl. XL, figs. 55-57), and young ova (Pl. XL, figs. 60, 61). Their division is similar to the division of the pre-flagellate stages, and requires no special description. Aggregation rosettes are formed (Pl. XXXIX, fig. 50).

Multiple division takes place in the deposited ova as in the early stages in the haemocoelic fluid. These forms, the result of multiple division, are multi-nucleated masses of protoplasm, and have been termed 'plasmodial forms' (Pl. XL, figs. 60, 61).

THE HEREDITARY INFECTION

The proofs of the occurrence of hereditary infection may be studied by the examination of living specimens and stained preparations. The process of events in the ovarian system is as follows:

About the time of oviposition, and during this event, the adult Crithidia pass by means of the haemocoelic fluid to the ovary and oviducts.

A large number of the crithidial forms here begin the post-flagellate ovarian stage of their existence in the walls (Pl. XXXIX, figs. 45-54) and cells (Pl. XL, figs. 55-57) of these organs. This post-flagellate or ovarian stage begins with the absorption of the flagella and the gradual rounding off of the parasites (Pl. XXXIX, figs. 45, 46) to Leishmania-like forms (figs. 47-49). A very large number of the parasites perform this evolution (fig. 50). A considerable number of the adult Crithidia, however, pierce the walls of the oviducts, preferably high up where the oval capsules are soft. Here they proceed to pierce the walls of the ova (Pl. XL, fig. 58), more than one parasite often entering the same ovum (Pl. XL, fig. 59). The aflagellar end is the foremost end in entering. A boring motion may now be observed, the flagellum describing a propeller-like movement, the whole organism being screwed forward, the undulating membrane forming the thread of the screw. Another motion has also been observed in the ovarian forms. After rounding of the protoplasm of the flagellar end, a circular motion has been noted which occurs previous to the disappearance of the flagellum and formation of the resting

bodies. Once within the egg the rounding-up process described above takes place, and the Leishmania-like stage results. Division of these small forms may be found in the ova (Pl. XL, figs. 60, 61), which ultimately break up into small resting bodies. A considerable number of the ova of infected ticks dry up and die. Generally the later deposited ova contain the resting forms; the ova deposited early in oviposition are usually non-infected.

SUMMARY

1. *Crithidia hyalommae* is a flagellate parasite occurring in the haemocoelic fluid, salivary glands, ovary, oviducts and ova of *Hyalomma aegyptium*, the common cattle tick of the Anglo-Egyptian Sudan.

2. The parasite has four periods in its life-cycle: a pre-flagellate stage (Pl. XXXVIII, figs. 1-8) passed chiefly in the haemocoelic fluid, a flagellate stage in the haemocoelic fluid (Pls. XXXVIII, XXXIX, figs. 9-39) and in the ovary and oviducts (Pl. XXXIX, fig. 50 and Pl. XL, figs. 58, 59), a post-flagellate stage (Pl. XXXIX, figs. 40-44) in the haemocoelic fluid, and a post-flagellate or ovarian stage in the ovary and oviducts (Pl. XXXVIII, XXXIX, figs. 45-54), ovarian cells (Pl. XL, figs. 55-57), and ova (Pl. XL, figs. 60, 61).

3. The pre-flagellate stage is of short duration. The parasites are small rounded or oval bodies from $4\ \mu$ to $11\ \mu$ broad, and from $4\ \mu$ to $12\ \mu$ long. Their protoplasm is reticulate or granular, depending on division. They contain a nucleus and a rod-shaped blepharoplast. Division of the very earliest forms takes place (Pl. XXXVIII, figs. 5-8).

4. The flagellate forms vary from $26\ \mu$ to $50\ \mu$ in length and from $2\ \mu$ to $2.5\ \mu$ in breadth.

The protoplasm is reticulate. The consistency of the nucleus varies with division. The rod (Pl. XXXIX, fig. 28a) or diplosomic blepharoplast (fig. 34) is usually situated on the flagellar side of the nucleus, but its position varies at times, depending on the phase of division. The flagellum arises in the neighbourhood of the blepharoplast, but not from it.

5. The undulating membrane and flagellum are both well marked. The undulating membrane assists considerably in motion.

The flagellum forms the border of the undulating membrane, and continues free from the protoplasmic body for a considerable distance.

A basal granule has been noted (Pl. XXXIX, fig. 31).

6. The post-flagellate and ovarian stages occur in the haemocoelic fluid, ovary and oviducts. A superfluity of the ovarian forms seems to be present, a large number of them entering a resting stage in the ovarian cells and tissues.

7. The ovarian stages of *Crithidia hyalommae* serve for the hereditary transmission of the parasite. The flagellates pass by means of the haemocoelic fluid to the ovaries and oviducts; here they pierce the ova, the aflagellar end of the parasite being anterior. Within the ova the flagellates round up and undergo subsequent division with the production of 'plasmodial' forms.

8. Multiplication of *C. hyalommae* by longitudinal division takes place in all its stages.

9. Infection of *Hyalomma aegyptium* by *Crithidia hyalommae* in the adult is purely a hereditary infection.

10. The flagellate is a strictly parasitic flagellate of the tick, and is non-pathogenic to the tick host.

ACKNOWLEDGMENTS

I wish to thank Dr. Balfour, the recent Director of these Laboratories, and Dr. Chalmers, the present Director, for their valuable assistance in both work and text; also Dr. Fantham, whose experience in this line of research is well known, for his valuable advice in the text and aid with the drawings. To Dr. Bousfield and his able staff I am indebted for a plentiful supply of working material.

KHARTOUM,

September 27th, 1913.

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EXPLANATION OF PLATES

PLATE XXXVIII

All figures were outlined with the Abbé-Zeiss camera lucida, after magnification with a 2 mm. apochromatic objective (Zeiss) with No. 12 compensating ocular.

The magnification of the figures is approximately 2,000 diameters.

- Figs. 1-4. Early pre-flagellate forms of *Crithidia hyalommae* from the haemocoelic fluid.
- Fig. 5. Young dividing form from haemocoelic fluid.
- Fig. 6. Plasmodial form from haemocoelic fluid.
- Figs. 7-8. Young forms from haemocoelic fluid.
- Figs. 9-10. Rounded form showing flagellum.
- Figs. 11-12. Early flagellates in haemocoelic fluid.
- Figs. 13-16. Division phases in early flagellates.
- Fig. 17. Early flagellate in haemocoelic fluid.
- Figs. 18-19. Division rosettes, haemocoelic fluid.
- Figs. 20-29. Development to full grown *Crithidia*, haemocoelic fluid.

PLATE XXXIX

- Fig. 28a. Fully grown Crithidia from haemocoelic fluid showing rod-shaped blepharoplast.
- Fig. 29a. Fully grown flagellate from haemocoelic fluid showing blepharoplast on flagellar side of nucleus.
- Figs. 31-33. Showing different positions of blepharoplast.
- Figs. 34-36. Commencing division of blepharoplast. Fig. 34 shows diplosome blepharoplast. Fig. 36. Parasite showing nuclear membrane and chromatic band from haemocoelic fluid.
- Figs. 30, 37, 38, 39. Stages in longitudinal division from haemocoelic fluid.
- Figs. 40-44. Post-flagellate stages in haemocoelic fluid.
- Figs. 45-49. Post-flagellate or ovarian stage in the ovary and oviducts.
- Fig. 50. Shows aggregation rosette of ovarian stages in walls of oviduct.
- Figs. 51-52. Division stages of Leishmania-like forms of ovary.

PLATE XL

Figs. 53-54. Division stages of ovarian forms of *Crithidia hyalommae*.

Figs. 55-57. Resting and division stages in ovarian cells.

Fig. 58. Entry of adult *Crithidia* into wall of oviduct.

Fig. 59. Perforation of egg by adult *Crithidia*.

Figs. 60-61. Plasmodial forms in deposited ova.

Fig. 62. Early form (rare) in salivary gland.